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DIAGNOSIS OF TUBERCULOSIS BY COMPLEMENT FIXATION, WITH SPECIAL REFERENCE TO BOVINE TUBERCULOSIS

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INTRODUCTION

Ever since the nature of tuberculosis has been established, the diagnosis of the disease in living animals as well as in man has caused considerable difficulty. Therefore it is not at all surprising that the efforts of the investigators were directed to devise means by which this most destructive disease could be readily and with certainty diagnosed. With the discovery of tuberculin a great advancement was made in this direction, especially with regard to its diagnostic value in animals. Nevertheless its shortcomings have been recognized, not because of its being unreliable but on account of the laboriousness of its application and the possibility of the so-called "doping" of the animals for the purpose of preventing a reaction. This practice is known to have been quite extensively employed in this country and in Germany, where the authorities were forced to abandon the recognition of this wonderful test on border importation as a result of the frequent frauds. In the search for other reliable means for diagnosis other allergic tests have been employed, such as the ophthalmic, cutaneous, intradermal, and intrapalpebral tests; but while favorable reports have been made on these various tests by individual investigators, as a whole they do not come up to the reliability of the subcutaneous test when it is scrupulously carried out.

The success obtained with the various biological tests for the diagnosis of infectious diseases directed attention toward their application in tuberculosis. Of the different biological determinations the agglutination and precipitation tests held out no encouragement for success. On the other hand the good results obtained with the complement fixation method for

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the diagnosis of diseases of animals, such as glanders, dourine, contagious abortion, Malta fever, etc., seemed to warrant further investigations for establishing the value of the test in tuberculosis, especially since the published results on similar investigations are very contradictory.

HISTORICAL RÉSUMÉ

It is well known that the first practical application of the Bordet-Gengou (6)[1] reaction was undertaken by Widal and Lesourd (40), who showed that antibodies of typhoid infections may be established much earlier in the serum of the patient by means of the complement fixation than with the agglutination method. Notwithstanding these clinical observations and the great possibilities for the Bordet-Gengou reaction, it was neglected for years until it was again taken up by Moreschi (29). This theoretical conception as to the reaction was not acceptable, especially since the investigations of Wassermann (37), Bruck (9), Liefmann (25), and Citron (12) showed, and later Moreschi himself recognized, that his former views could not be supported by the results of the subsequent investigations. According to Moreschi's theory the Bordet-Gengou reaction represented a precipitation reaction, and the formed precipitin was mechanically carried down by the complement. When he later observed that at times even strongly precipitating serums failed to give the reaction, he recognized the unsoundness of his theory.

Neisser and Sachs (31) also undertook investigations with the Bordet-Gengou reaction, endeavoring to employ it for the differentiation of albumens. Since their findings were irregular and contradictory, Wassermann carried on investigations to establish whether it would be possible to differentiate dissolved bacterial albumens with the aid of the test. Wassermann further aimed to prove that before the state of antibody production there are probably other intermediate products in the circulation which might be detected by means of complement fixation as applied by Neisser and Sachs for the differentiation of albumens. Wassermann failed to demonstrate the presence of such intermediate bodies; and in collaboration with Bruck (37) he directed his attention toward establishing the presence of an antigen in diseased organs, using lungs, glands, and other diseased tissues affected with tuberculosis. Extracts of these organs were prepared and used against serums of tuberculous individuals, with the result that such serums did not fix the complement unless the patient had been treated with tuberculin for a certain period. The reaction obtained was due to the formation of antibodies as a result of the injection of tuberculin. Wassermann called these antibodies "antituberculin." Citron (12), on the other hand, named them "antituberculin amboceptors."

The findings of Wassermann and Bruck were questioned by various investigators. Weil and Nakayama (38) and Morgenroth and Rabino-

[1] Reference is made by number to "Literature cited," p. 18–20.

witz (30) were the first who disagreed with their conclusions. Citron, while agreeing with the principles of the Wassermann-Bruck conception, continued the investigations for the purpose of determining whether it would be possible to establish antibodies in the blood of tuberculous individuals, though they were not treated with tuberculin. Employing Wassermann's method, he obtained in 9 untreated cases of tuberculosis 2 positive and 7 negative results; whereas in 14 treated cases, 8 were positive and 6 negative. Ludke (27) also confirmed the essentials of the Wassermann-Bruck work, as he found antituberculin present in tuberculous tissue in man in 3 out of 5 cases. He also found it in the serum in 17 out of 31 treated cases and in 2 advanced cases out of 10 untreated cases. In 18 cases, none of which were tuberculous, negative results were obtained in all instances. Ludke also observed that antituberculin was present, especially in cases where the administration of tuberculin was not followed by a systemic reaction. In the cases in which this injection was followed by a reaction he failed to obtain any fixation. In 8 cases tested both before and after the administration of tuberculin antibodies were found in 7 after injection. In 3 cases affected with diseases other than tuberculosis the injection of tuberculin was not followed by the appearance of antituberculin.

Wolff-Eisner (42) denied the specificity of the reaction because they found that the serums of patients suffering from pneumonia, typhoid fever, or lues gave a similar reaction to those affected with tuberculosis. Laub and Novotny (23) also questioned the usefulness of the reaction, as they found that the results were very irregular, tuberculous patients giving the same proportion of reactions as healthy individuals. Cohn (13), on the other hand, established the usefulness of the reaction, provided the test is carried out with the utmost care, including the use of all controls, in which case the presence of antibodies may be demonstrated not only by the tuberculin-treated individuals but also in progressive cases. A number of investigators, as M. Wolff and Mühsam (41), Czastka (15), Bérmbach (2), Weil and Strauss (39), claimed to have been able to obtain favorable reactions in 20 to 50 per cent of their tests. On the other hand, the followers of Escherich (20) declared that at no time was it possible to determine antibodies in tuberculous children. Schlossmann (34) and Engel and Bauer (19) confirmed these views. Engel and Bauer, investigating a series of tuberculous affections in children, failed to find specific antibodies either in the serum of healthy or in infected children. In all cases of tuberculosis which were specifically treated, antibodies developed. They further found that the antibodies increased in proportion to the injections of tuberculin and diminished after discontinuing the treatment. Frugoni (21) and Szabóky (36) also concluded that the complement fixation in tuberculosis is of no value for diagnostic purposes, which view was also held by Bach (1) with regard to animal tuberculosis.

These contradictory results stimulated the investigators to further work. Dieterlen (17), continuing the experiments of Christian and Rosenblat (11), found that normal animal serums do not contain antibodies but that spontaneously or artifically infected animal serums possess them. Szabóky stated that rabbits always possess antibodies against tuberculin, but that they disappear whenever the animal becomes tuberculous. In guinea pigs such antibodies are invariably absent. Löwenstein (26), employing old and new tuberculin as antigen, found antibodies present in a high percentage of tuberculous individuals in advanced stages of the disease. In cases of recent infections they were almost invariably absent. In cases treated with tuberculin they usually disappear for a short time and reappear about the eighth day after the injection and subsequently increase in amount. Ruppel (33) noted a marked rise in the quantity of antibodies in the serums of bovines inoculated with human tubercle bacilli and treated with tuberculin.

Emery (18) employed sterile bacillary emulsion as antigen, using the complement present in the serum to be tested, and human red blood corpuscles, also a special technique by varying the time during which the ingredients remained together at 38° C. He obtained 82 per cent of positive reactions in 56 cases of tuberculosis and 17.6 per cent positive reactions in 34 nontuberculous cases.

Porter (32) investigated the sera of cattle and obtained complement fixation in normal as well as in tuberculous cases, the reaction being more characteristic in the advanced cases than in the early stage of the disease.

Wyschelewsky (43), also working with cattle, confirmed Porter's results. Both observers concluded, however, that this method is not suitable for the differentiation of latent cases from the more progressive ones.

Deilmann (16) compared the various extracts from the tubercle bacilli such as proteins, fatty acid, and tuberculo-nastin individually and also each in turn with tuberculin for their antigenic properties, and concluded that all the extracts act as antigens, but none are as suitable in this respect as an emulsion of the entire bacillus or as tuberculin.

Calmette and Massol (10) and also Letulle (24) used as antigens watery extracts of bovine tubercle bacilli and bacilli macerated in peptone. They followed a more minute technique by adding varying doses of complement and succeeded in demonstrating antibodies in a large percentage of tuberculous cases. They consider that antibodies are not invariably present, because in many cases a substance is present in the serum which inhibits the reaction. They further obtained the most marked reactions in cases of a slow progressive character, while in acute cases the results were more often negative.

Hammer (22), in using a mixture of tuberculous synovial membrane and tuberculin as antigen, obtained out of 96 cases, 50 positive and 46

negative reactions, of which 48 were found positive and 48 negative on post-mortem examination. Exception was taken to his findings by Bierbaum and Berdel (5), who concluded that alcoholic or aceton extracts of tuberculous tissues as antigens do not confirm the results of Hammer. Of all antigens the greatest support was given to the one prepared by Besredka (3, 4). It is essentially a tuberculin prepared in glycerin and peptone-free egg medium. Many investigators reported favorably on the action of this antigen, and the only objection against it was its nonspecific reaction obtained in cases of nontuberculous syphilitic individuals. Bronfenbrenner and Rockman (8) concluded with regard to Besredka's antigen that different samples of his tuberculin, though prepared apparently in an identical manner, may differ in their specific values. Thus, different samples were found to vary in the amount of lipins they contained. It is necessary to free the samples of tuberculin entirely from the lipins present when used for the complement deviation test. Besredka's antigen seems to give the best results in the diagnosis of tuberculosis by means of complement fixation, and though the test is positive in a certain number of nontuberculous cases the reaction seems to be specific. However, some investigators experienced difficulties in preparing the antigen according to the directions given by Prof. Besredka.

More recently Stimson (35) carried on extensive investigations on this subject and employed an alcoholic precipitate of the tubercle bacillus emulsion filtrate as antigen, obtaining promising results. Favorable results were further reported by Craig (14) with an antigen prepared from a liquid egg-medium growth material, and by Miller and Zinsser (28) with a bacterial emulsion in hypotonic salt solution. These authors claim good results, especially for differentiation between active and nonactive infections.

The foregoing historical outline furnishes ample evidence that strong efforts were made toward devising a method by which the complement fixation might be utilized for the diagnosis of tuberculosis.

ANTIGENS

In the foregoing literature mention was made of the various antigens which were used for the diagnosis of tuberculosis by means of complement fixation. These antigens may be classified into three groups, according to their contents:

1. Tuberculin.
2. Emulsion of tubercle bacilli.
3. Extracts of tuberculous tissues.

In consideration of the failures of former investigators with various antigens containing the specific products of the germ and of the disease, it suggested itself that an antigen containing the toxic products together with bacteria would probably combine the active elements for the phenomena of the reaction. Therefore experiments were undertaken with

an antigen consisting of tuberculin and tubercle bacilli in varying proportions.

In the preparation of tuberculin the liquid medium contains glycerin in an amount varying from 5 to 7 per cent. Besredka, in preparing his egg medium, eliminated entirely the glycerin. In the following experiments the ordinary old tuberculin freed from its glycerin contents has been utilized. The bacillary emulsion used by different authors was prepared in various ways. The emulsions consisted of finely triturated bacteria suspended in normal or hypotonic salt solution. Alcoholic precipitates of bacterial extract were also utilized (14, 35). For the purposes of the experiments only defatted organisms were employed, because it was assumed that the waxy capsule of the bacilli might prevent the extraction of the endotoxins; and furthermore, it was desired to avoid nonspecific reactions due to the presence of lipins. Therefore the antigen was composed essentially of concentrated tuberculin and the defatted organisms. At the same time comparative tests were carried out with other antigens. The following are especially mentioned:

1. Emulsions of tubercle bacilli in normal or hypotonic salt solutions.
2. Emulsions of defatted tubercle bacilli in normal or hypotonic solutions.
3. Unheated nonconcentrated tuberculin freed from bacilli by passage through Berkefeld filters.
4. Unheated nonconcentrated tuberculin freed from bacilli by passage through Berkefeld filters fortified with aceton insoluble lipoids, as prepared by Noguchi. The aceton insoluble lipoids were previously standardized against known positive syphilitic sera to establish its titer. One unit of the antigen was added to 10 c. c. of the tuberculin mentioned and this mixture was then used as a menstruum in the emulsification of the bacilli.
5. Besredka's antigen—(a) as received through the courtesy of Prof. Besredka, (b) a modification of the same, (c) a modification of the same unheated and freed from bacilli by passage through Berkefeld filters.

The modified Besredka's medium was prepared as follows: Twenty c. c. each of the white and the yolk of an egg were thoroughly beaten in an automatic egg beater, and to the whipped material a solution of Liebig's meat extract (3:1,000) in distilled water was gradually added while the mixture was continuously beaten. A sufficient meat-extract infusion was used to make up the whole to 1,000 c. c. The emulsion was strained through cotton and heated to the boiling point, then strained again, and after the addition of 0.5 per cent of sodium chlorid was carefully neutralized, heated again and strained, and neutralized if necessary. To the neutral medium sufficient normal sodium-hydroxid solution was added to make the medium of 0.2 alkalinity. This medium, to which neither peptone nor glycerin was added, was then autoclaved at 115° C. for 20 minutes, and after cooling was kept at 37° for 48 hours for observation as to sterility. Human and bovine cultures containing a 30-days' growth of the tubercle bacilli, heated for 20 minutes at 115° and filtered, were used as antigen. This antigen was standardized in the usual manner.

EXPERIMENTAL WORK

PREPARATION OF THE ANTIGENS EMPLOYED

Material used: Old tuberculin; saturated solution of ammonium chlorid; Schulke and Schleich parchment paper.

To an amount of old tuberculin in an Erlenmeyer flask of convenient size sufficient saturated solution of ammonium chlorid is added to supersaturate the tuberculin. The mixture is stirred well with a glass rod and set aside for 48 hours. The fluid is carefully poured off from the precipitate which has settled at the bottom of the flask. The liquid is centrifuged at a high rate of speed and the sediment collected, which consists of a resinous sticky mass, dark brown in color, is readily dissolved in a small amount of salt solution, and is then transferred to the dialyzing (parchment paper) bag. The sediment contained in the Erlenmeyer flask is added to it and the material is then dialyzed for 8 consecutive days. The content of the bag is then tested for the presence of ammonium chlorid, and if no reaction for ammonia is obtained the substance is removed, filtered, and the filtrate evaporated to dryness over a water bath at 60° C.

The residue was scraped from the evaporating dish, and the chocolate-brown colored powder collected, placed in a bottle which was provided with a well-fitting stopper, and the precipitate kept in a cool place, protected from light. The amount of precipitate obtained corresponds to about 0.8 to 1 per cent.

It should be emphasized that the preparation and handling of the precipitate must be carried out under precautions to prevent the inhalation of the pulverized precipitate. Even slight amounts may cause disagreeable symptoms. These are manifested in severe headaches and chills, accompanied by high fever, the temperature ranging between 102° and 104° F. for two or three consecutive days; pulmonary symptoms resembling those of a lobular pneumonia and profuse night sweats; respiration sometimes difficult and painful; pains in the back are always present; the urine is dark-colored; micturition frequent and scanty. For protection it is advisable to cover the evaporating dish during the pulverization with several layers of cheesecloth moistened with a 5 per cent phenol solution.

The material used for the preparation of defatted organisms was as follows: Surface growth of a bouillon culture of tubercle bacilli of both human and bovine strains; alcohol, 95 per cent; ether.

The tubercle bacilli are macerated for seven days in 95 per cent ethyl alcohol in a closed vessel. The alcohol is then removed and the bacteria are macerated with 50 c. c. ethyl ether for six hours, shaking the mixture frequently. The ether is poured off and the bacilli are shaken two more times for one-half hour each time with 25 c. c. of ether. The bacilli are then collected, dried in vacuum over sulphuric acid, and

finally kept in a well-stoppered vial in a cool place protected from the light.

Standardization of Antigen for Final Test

Complement.—In the early work of the writers on the complement fixation in tuberculosis the intention was to use one unit of the accurately standardized complement. The titer was determined by establishing the smallest amount of complement which caused complete hemolysis within one-half hour of incubation. It was soon observed, however, that this amount was insufficient to establish the proper amount of complement necessary to obtain the best results. It was found that a 5 per cent dilution proved most suitable, and accordingly that proportion was employed throughout this work.

Two units of anti-sheep-blood hemolysin were used throughout.

Red sheep-blood corpuscles.—Various dilutions of the suspension were tested, and the most satisfactory results were obtained with a 3.5 per cent sheep-blood corpuscle.

Salt solution.—While the normal physiologic salt solution was used in most cases, tests were also made with 0.75 per cent. It was noted that by employing the hypotonic solution the reactions developed more rapidly.

Serums used for standardization of antigens.—The serums were obtained from tuberculin-tested animals. At least 12 months elapsed from the time of the last negative test. The inactivation was carried on at 58° C. for one-half hour and 0.2 c. c. was the amount used in the test in every tube.

Two methods were used in the standardization of the antigen.

Method 1: Standardization of Antigen

Thirty mgm. of defatted and dried tubercle bacilli were pulverized in a 12-cm. mortar. The powder was then emulsified in a solution containing 50 mgm. of dried tuberculin in 10 c. c. of 0.6 per cent salt solution. This emulsion served as the antigen and was titered for its antigenic, anticomplementary, and hemolytic properties. The titration was conducted according to Tables I to III.

Table I.—*Titration for antigenic dose*

Tube No.	Antigen.	Inactivated positive serum.	Complement, 5 per cent.	Physiological salt solution.	Incubation period.	Hemolysin.	Erythrocytes, 3.5 per cent.	Incubation period.	Degree of hemolysis.
	C. c.	*C. c.*	*C. c.*				*C. c.*		
1	0.5	0.2	1	Sufficient quantity to make 2 c. c.	1 hour.	2 units contained.	1	1 hour.	None.
2	.4	.2	1			.....do.....	1		Do.
3	.3	.2	1			.....do.....	1		Do.
4	.2	.2	1			.....do.....	1		Do.
5	.1	.2	1			.....do.....	1		Incomplete.
6	.05	.2	1			.....do.....	1		Complete.
7	None.	.2	1			.....do.....	1		Do.
8	.1	None.	1			.....do.....	1		Do.

TABLE II.—*Titration for anticomplementary dose*

Tube No.	Antigen.	Inactivated normal serum.	Complement, 5 per cent.	Physiological salt solution.	Incubation period.	Hemolysin.	Erythrocytes, 3.5 per cent	Incubation period.	Degree of hemolysis.
	C. c.	*C. c.*	*C. c.*				*C. c.*		
1	0.5	0.2	1	Sufficient quantity to make 2 c. c.	1 hour.	1 c. c. containing 2 units.	1	1 hour.	Incomplete.
2	.4	.2	1			.....do.....	1		Complete.
3	.3	.2	1			.....do.....	1		Do.
4	.2	.2	1			.....do.....	1		Do.
5	.1	.2	1			.....do.....	1		Do.
6	.005	.2	1			.....do.....	1		Do.
7	None.	.2	1			.....do.....	1		Do.
8	.1	None.	.....			.....do.....	1		Do.

TABLE III.—*Titration for hemolytic properties*

Tube No.	Antigen.	Inactivated normal serum.	Erythrocytes.	Physiological salt solution.	Incubation.	Degree of hemolysis.
	C. c.	*C. c.*	*C. c.*			
1	0.6	0.2	1	Sufficient quantity to make 3 c. c.	1 hour.	Very faint.
2	.5	.2	1			None.
3	.4	.2	1			Do.
4	.3	.2	1			Do.
5	.2	.2	1			Do.
6	.1	.2	1			Do.
7	.05	.2	1			Do.
8	None.	.....	.....			Do.
9	.3	None.	.....			

METHOD 2: STANDARDIZATION OF BACILLARY EMULSION WITH A FIXED AMOUNT OF TUBERCULIN PRECIPITATE

Solutions containing 10, 20, 30, 40, etc., mgm., in 10 c. c. of a 0.6 per cent solution of sodium chlorid were prepared from the tuberculin precipitate described under the preparation of antigen. Tests were undertaken with the various dilutions, 0.1 c. c. of each serving as a basic amount of the antigen to which increasing amounts of the bacillary emulsion had been added in the different tubes. Tables IV to VI demonstrate the procedure of the tests.

TABLE IV.—*Titration for antigenic dose*

Dose No.	10 mgm. tuberculin precipitate in 10 c. c. sodium-chlorid solution.[a]	Bacillary emulsion.	Inactivated positive serum.	Complement, 5 per cent.	Physiological salt solution.	Incubation.	Hemolysin (1 unit = 0.5 c. c.)	Erythrocytes, 3.5 per cent	Incubation.	Degree of hemolysis.
	C. c.	*C. c.*	*C. c.*	*C. c.*				*C. c.*		
1	[b] 0.1	0.40	0.20	1	Sufficient quantity to make 2 c. c.	1 hour.	2 units.	1	1 hour.	None.
2	[b] .1	.30	.20	1			...do.....	1		Do.
3	[b] .1	.20	.20	1			...do.....	1		Do.
4	.1	.15	.20	1			...do.....	1		Do.
5	.1	.10	.20	1			...do.....	1		Incomplete.
6	.1	.05	.20	1			...do.....	1		Complete.
7	.1	None.	.20	1			...do.....	1		Do.
8	.1	.10	None.	.....			...do.....	1		Do.

[a] Similar tests were made with solutions containing 20, 30, 40, and 50 mgm. of tuberculin precipitate in 10 c. c. of a solution of sodium chlorid.

[b] In solutions 1, 2, and 3, four mgm. per cubic centimeter were used.

TABLE V.—*Titration for anticomplementary dose*

Dose No.	Tuberculin precipitate solution.	Bacillary emulsion.	Inactivated normal serum.	Complement, 5 per cent.	Physiological salt solution.	Incubation period.	Hemolysin (1 unit = 0.5 c. c.)	Erythrocytes, 3.5 per cent	Incubation period.	Degree of hemolysis.
	C. c.	*C. c.*	*C. c.*	*C. c.*	Sufficient quantity to make 2 c. c.	1 hour.	2 units	*C. c.* 1	1 hour.	Incomplete.
1	0.1	0.40	0.2	1			...do	1		Complete.
2	.1	.30	.2	1			...do	1		Do.
3	.1	.20	.2	1			...do	1		Do.
4	.1	.15	.2	1			...do	1		Do.
5	.1	.10	.2	1			...do	1		Do.
6	.1	.05	.2	1			...do	1		Do.
7	.1	None.	.2	1			...do	1		Do.
8	.1	.1	None.	1			...do	1		Do.

TABLE VI.—*Titration for hemolytic properties*

Tube No.	Solution of tuberculin precipitate.	Bacillary emulsion.	Inactivated serum.	Erythrocytes, 3.5 per cent.	Physiological salt solution.	Incubation period.	Degree of hemolysis.
	C. c.	*C. c.*	*C. c.*	*C. c.*	Sufficient quantity to make 3 c. c.	1 hour.	
1	0.1	0.40	0.2	1			None.
2	.1	.30	.2	1			Do.
3	.1	.20	.2	1			Do.
4	.1	.15	.2	1			Do.
5	.1	.10	.2	1			Do.
6	.1	.05	.2	1			Do.
7	.1	None.	.2	1			Do.
8	.1	.15	None.	1			Do.
9	.1	None.	.2	.....			Do.

Table VII demonstrates the procedure employed in the final test and also the controls used.

TABLE VII.—*The final test*

Tube No.	Antigen.	Inactivated serum 0.2 c. c.	Complement, 5 per cent.	Physiological salt solution.	Incubation period at 37° C.	Hemolysin (1 unit = 0.5 c. c.).	Erythrocytes, 3.5 per cent	Incubation period at 37° C.	Degree of hemolysis.
Positive controls:	*Unit.*		*C. c.*	Sufficient quantity to make 2 c. c.	1 hour.	*Units.*	*C. c.*	1 hour.	
1	1	+ Serum	1			2	1		None.
2	1½	...do	1			2			Do.
3		...do	1			2	1		Perfect.
Negative controls:									
4	1	− serum	1			2	1		Do.
5	1½	...do	1			2	1		Do.
6		...do	1			2	1		Do.
Suspected serum:									
7	1	Suspected serum	1			2	1		?
8	1½	...do	1			2	1		?
9		...do	1			2	1		?
Controls:									
10	1½	...do	1			2	1		Perfect.
11			1			2	1		Do.

SOURCE AND TESTING OF SAMPLES

The material for testing was obtained from animals slaughtered at the Baltimore and the Washington slaughterhouses and from animals which were subjected to periodical tuberculin tests. The samples obtained from the abattoirs were collected in sterile glass jars or test tubes. Every sample of blood was accompanied by a post-mortem report from the veterinary inspector, describing in detail the condition of the animals and also the character and extent of the lesions. Figure 1 is a repro-

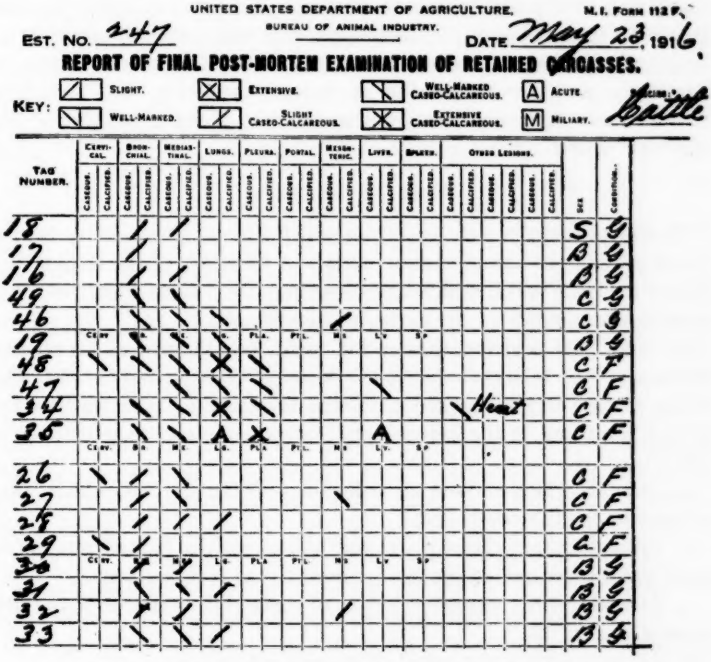
UNITED STATES DEPARTMENT OF AGRICULTURE. M. I. Form 112 F.
BUREAU OF ANIMAL INDUSTRY.
EST. NO. 247 DATE May 23, 1916.
REPORT OF FINAL POST-MORTEM EXAMINATION OF RETAINED CARCASSES.
KEY: Slight. Extensive. Well-Marked Caseo-Calcareous. A Acute. Well-Marked. Slight Caseo-Calcareous. Extensive Caseo-Calcareous. M Miliary.
Inspector Battle
Tag Number. Cervical. Bronchial. Mediastinal. Lungs. Pleura. Portal. Mesenteric. Liver. Spleen. Other Lesions. Sex. Condition.
Caseous. Calcified.
18 1 1 S G
17 1 B G
16 1 1 B G
49 1 1 C G
46 1 1 1 C G
19 Cerv. Bron. Med. Lu. Pl. Port. Mes. Liv. Sp. B G
48 1 1 1 X 1 C F
47 1 1 C F
34 1 1 X 1 Heart C F
35 1 1 A X C F
Cerv. Bron. Med. Lu. Pl. Port. Mes. Liv. Sp.
26 1 1 1 C F
27 1 1 C F
28 1 1 1 C F
29 1 1 C F
30 Cerv. Bron. Med. Lu. Pl. Port. Mes. Liv. Sp. B G
31 1 1 1 B G
32 1 1 1 B G
33 1 1 1 B G

FIG. 1.—Specimen of post-mortem report on retained carcasses in Federal meat inspection

duction of a post-mortem report showing the character of the lesions of animals the serums of which were subjected to the complement-fixation test.

The samples of blood obtained from animals prior to the injection of tuberculin were conveyed from the jugular direct into sterile bottles. All samples of blood were delivered at the laboratory without any delay. After the breaking up of the clots, they were centrifuged; and from the supernatant clear serum a sufficient quantity was taken for the test.

Some tests were performed with active serums, but without success, as the results in most instances showed an anticomplementary action.

Inactivation at 55° for one-half hour was not uniformly satisfactory; it was found that an inactivation of 58° gave the best results. Buck's method of inactivation (unpublished) was also employed, in which equal parts of salt solution are added to the serums and the inactivation is carried at 62°. Both methods were uniformly satisfactory, though in a few instances Buck's method appeared to have an advantage over the former. The quantity of the inactivated serum used for the test was 0.2 c. c.

Table VII describes in detail the procedure of the test, in which the incubation time is designated as one hour. In order to obtain the best results in the preliminary experiments, care was taken to establish the length of time the racks should be kept at incubation before adding the hemolysin and the suspension of blood corpuscles. By subjecting various racks to incubations from one to four hours it was found that one-hour periods served to the best advantage.

RESULTS OF TESTS

In all 958 samples were tested, of which 816 samples were of bovine, 120 of porcine, and 22 of human origin. A summary of the tests is contained in Table VIII, which also describes the lesions found on post-mortem examination of the cattle in the abattoirs.

In order that a clearer conception might be obtained as to whether there exists any relation between the degree of reaction and the character of the disease it was deemed advisable to separate the cases into five groups according to the lesions found on post-mortem examination, as follows:

1. Animals which showed no lesions of tuberculosis and which failed to react to the tuberculin test.
2. Animals which showed arrested lesions with a limited number of small slight caseo-calcareous foci confined to the lymph glands.
3. Animals with progressive lesions of a glandular type involving also some of the organs.
4. Animals with well-marked or generalized lesions.
5. Acute and miliary tuberculosis.

In considering the results of these tests there appears to be no constancy in the reaction; and furthermore, the degree of the reaction appears to have no relation to the extent of the disease. It was also noted that the condition of the animal has apparently no effect on the reaction, which as a matter of fact is also the case in the subcutaneous tuberculin test, although in the latter advanced generalized cases may fail to respond.

The degree of the fixation is also irregular, since the slight positive reactions (+) do not occur proportionally with a greater frequency in mild cases than the complete fixation (+ + + +). This must be considered a great disadvantage, as a slight reaction might be overlooked or might be obtained as a result of the slightest neglect in the standardization of the

antigen. It may be possible that future investigation will establish a greater accuracy of this test, but the results obtained warrant the statement that the comparative tests with various antigens have proved that an antigen prepared from a bacillary emulsion and tuberculin precipitate is most suitable for the completement-fixation test.

TABLE VIII.—*Summary of the tests conducted with an antigen prepared from a bacillary emulsion and tuberculin precipitate*

Bovine serum.	Total number.	Degree of fixation.						Percentage.
		++++	+++	++	+	±	−	
Group 1: Serums from animals without lesions of tuberculosis and negative to the tuberculin test	320	3	2	10	13	17	275	85.94
Group 2: Serums from animals showing arrested lesions of tuberculosis	207	21	28	39	48	19	52	77.15
Group 3: Serums from animals with progressive lesions	79	11	16	23	16	5	8	83.56
Group 4: Serums from animals showing generalized, well marked, or extensive lesions	43	12	9	4	7	4	7	74.42
Group 5: Serums from animals showing acute or miliary lesions	31	9	4	4	10	1	3	87.1
Total	680	56	59	80	94	46	345	81.63

As indicated in Table VIII, out of 320 negative serums a total of only 275, or 85.9 per cent, proved distinctly negative on the test; whereas of 360 positive serums 290, or 80.5 per cent, were positive. Furthermore, of the affected animals 81, or 25.8 per cent, gave only slight positive reactions, and 80, or 7.6 per cent, were atypical. The grand total of 81.6 per cent of accurate reactions obtained is by no means sufficient to make the test practical for the control of the disease, especially since the allergic tests which we have at our command are more reliable.

No satisfactory explanation can be given for the failure of reactions in known tuberculous infections or for the positive reactions in apparently healthy individuals. In regard to this various theories have been advanced; but since there is no confirmatory data available for any of these, it appears superfluous to discuss them here. The fact, however, that there existed a possibility that some of the positive results in the negative cases might have been due to a previous tuberculinization of the animals can not be overlooked, since no history was available regarding the animals which furnished the serums. It is therefore probable that a number of the atypical and possibly also some of the positive reactions in the apparently healthy animals might have resulted from an injection of tuberculin, which to-day is quite extensively practiced for diagnostic purposes.

In order to establish whether a tuberculin injection would interfere with the results of the tests, two vigorous young animals were subcutaneously injected with 2 c. c. of Bureau of Animal Industry tuberculin. Blood samples were obtained from these animals prior to the injection and subjected to the complement-fixation test with negative results. Subsequent to the tuberculinization, blood was drawn twice weekly from them. On the fourth day following the injection a positive reaction was obtained; after 10 days the fixation was complete, persisting for four weeks; it was then followed by partial fixation, and completely subsided after six weeks. It is possible that in some animals the fixation might persist for a still longer period; therefore it is essential to determine whether an animal has been injected with tuberculin, and if so, how long ago, before judgment can be passed on the results of the complement-fixation test.

In connection with these experiments a peculiar phenomenon was observed in several instances. In using an antigen which contained a higher amount of precipitate than the standard established by titration, a reaction which might be designated as a reverse reaction followed—that is, instead of the expected positive reaction it proved negative, and vice versa.

While it is not intended to offer a theoretical explanation for this peculiarity, it serves to emphasize the necessity for a careful standardization of the antigen each time the test is undertaken. The apparent delicacy of the test in the diagnosis makes it essential to employ more than one known positive and known negative serum in standardizing the antigen. In the experiments cited the antigens were always standardized to a series of known positive and negative serums.

COMPARATIVE TESTS WITH VARIOUS ANTIGENS

The advantages of an antigen prepared by our method have already been described. In order, however, to establish its comparative value various other antigens were used and the following brief mention is made of the methods of their preparation and the results obtained.

For the antigen prepared from tubercle bacillary emulsion, dilutions were made from a stock emulsion in which 30 mgm. were suspended in each cubic centimeter of the emulsion. The stock suspension was never heated and was prepared by milling the bacilli in a porcelain mill for about 40 days. The dilution used as antigen consisted of 3 mgm. per cubic centimeter in either physiological salt solution or in hypotonic solution. It was observed that the emulsion made up with the normal physiological salt solution gave an excessive number of positive reactions, whereas the one prepared with the hypotonic solution gave much closer results. Nevertheless the comparative results proved this antigen to be unsatisfactory. Its greatest disadvantage proved to be that the difference between the

antigenic and the anticomplementary properties was of such short range that the reliability of the tests with this antigen had to be questioned.

The same experience was obtained with an antigen prepared from defatted organisms. The only advantage of this antigen as compared with the preceding one was noted to be in the shorter time in which the reaction took place, and in some instances it appeared more reliable. The precipitation method of Stimson (35) was also tried, but without much success. It was the intention also to employ the combined Besredka-Stimson method, as advocated by Craig (14), but this was discarded when a communication was received from Dr. Craig in which he stated that the antigen devised by him might be improved.

An antigen prepared from an unheated liquid medium and Berkefeld-filtered proved to be of no value. The same in combination with the bacteria gave results similar to the antigen prepared from tubercle bacillary emulsion and from defatted organisms; and when reinforced with lipoids, the reactions obtained were much more distinct. At the same time, however, many nonspecific reactions were obtained. According to Calmette (10), the serum of a tuberculous individual contains more lecithin than that of a healthy person. Whether this observation has any bearing on the more definite reaction obtained with antigens containing a lipoid substance or whether the more distinct reactions are due to changes of lipoidic bodies present in the serum can not be stated.

In using Besredka's antigen for comparative tests it was desirable to establish whether the nonspecific reaction reported by Bronfenbrenner (7) and Stimson (35) could be observed in tests conducted with serums of cattle. In these instances the presence of syphilis, which was assumed to be the cause of some atypical reactions, was excluded. Nevertheless similar disturbing factors were observed in the tests with bovine serums. It has been stated that some of the nonspecific reactions might have been due to the possibility that the serums originated from animals which had been previously tested with tuberculin. There were, however, nonspecific reactions obtained in animals belonging to a herd in which the tuberculin test had not been applied for at least one year, the animals of this herd having been proved free from tuberculosis for years by periodical testing. Table IX serves as a comparison as to the results obtained with Besredka's antigen and the one prepared by the writers from tuberculin precipitate and defatted organisms.

The number of cases tested and the results recorded in Table IX would not appear to justify any definite claims as to the comparative value of Besredka's antigen and the one prepared by the writers. However, in the course of these experiments numerous samples of serums were repeatedly tested with various antigens in order to establish their relative accuracy, and, based especially on these results, a considerable advantage appeared to be in favor of the antigen prepared from a bacillary emulsion and tuberculin precipitate.

TABLE IX.—*Summary of the tests conducted for comparative purposes with (I) the antigen of Besredka and (II) the antigen prepared from a bacillary emulsion and tuberculin precipitate*

Bovine serum.	Number of cases.	Degree of fixation.												Percentage.	
		++++		+++		++		+		±		−			
		I	II	I	II	I	II	I	II	I	II	I	II	I	II
Group 1: Serums from animals without lesions of tuberculosis and negative to the tuberculin test	91	2	.....	1	2	1	1	4	3	....	....	81	83	91.1	93.4
Group 2: Serums from animals showing arrested lesions of tuberculosis	35	2	.....	.....	....	1	....	12	19	4	7	16	9	42.80	54.29
Group 3: Serums from animals showing progressive lesions of tuberculosis	3	.....	.....	.....	....	....	....	2	3	1	....	....	....	66.67	100
Group 4: Serums from animals showing generalized well marked or extensive lesions	1	.....	.....	.....	....	....	....	....	....	....	....	1	1	.....	.....
Group 5: Serums from animals showing acute and miliary lesions of tuberculosis	6	.....	.....	.....	....	1	1	....	....	4	4	1	1	16.35	16.35
Total	136	4	.....	1	2	3	2	18	25	10	11	99	94	43.58	66.16

TESTS OF HOG SERUMS

Besides the testing of bovine serums, a limited number of tests were undertaken with hog serums. Most of these were obtained from abattoirs from hogs which on post-mortem examination revealed lesions of tuberculosis, irrespective of the extent of the disease. A desirable number of apparently healthy hogs were also included in the test. No detailed post-mortem charts accompanied the serums, they being only marked "positive" or "negative." A limited number of serums were also obtained from hogs which reacted to the intradermal tuberculin test and which on post-mortem examination proved to be affected with tuberculosis. In all, 120 hogs were tested.

The results of the complement-fixation test in tuberculous hogs were very irregular and much less reliable than in cattle. This might be attributed to the fact that tuberculosis in hogs as observed in meat inspection is limited in about 95 per cent of the infections to the lymph glands of the head and neck in the form of isolated foci, showing in most

instances a retrogressive tendency. Such moderate lesions might not induce the formation of sufficient antibodies in the animal to permit detection by the test.

TESTS OF HUMAN SERUMS

A limited number of human serums were also included in the tests in order to establish whether the results with a certain antigen would be more reliable with animal serum than with human serum. In all, 22 cases were tested. Most of the positive serums were obtained from patients of the Tuberculosis Hospital at Washington, D. C., through the courtesy of the authorities. These tests are summarized in Table X.

TABLE X.—*Summary of the tests on human serums conducted with an antigen prepared from a bacillary emulsion and tuberculin precipitate*

Human serum.	Number of cases.	Degree of fixation.						Percentage.
		++++	+++	++	+	±	−	
1. Apparently healthy	5	.....	.....	.....	.....	.....	5	100
2. Clinical symptoms present, sputum negative	4	.....	.....	.....	1	2	1	33
3. Clinical symptoms moderate, sputum positive	3	2	.....	.....	.....	.....	1	67
4. Clinical symptoms progressive, sputum positive	6	3	1	1	1	.....	.....	100
5. Clinical symptoms severe (cavities), sputum positive	4	.....	1	1	.....	.....	2	50
Total	22	5	2	2	2	2	7	70

From Table X it appears evident that the results are not more reliable than in bovine tuberculosis and also that the degree of the reaction has no bearing on the extent of the disease. However, it is recognized that the number of human cases tested does not warrant any definite conclusions.

CONCLUSIONS

(1) The complement-fixation test for the diagnosis of tuberculosis in cattle is not so reliable as the subcutaneous tuberculin test.

(2) Since a large proportion of the positive cases give only a faint reaction, it necessitates a very careful titration of the antigen and a most accurate observance of all of the details of the technique of the test.

(3) The amount of fixation can not be considered as an index to the extent of infection. Frequently samples from animals showing arrested, retrogressive lesions gave a more marked fixation than animals affected with acute, progressive, generalized tuberculosis.

(4) The complement-fixation test might be employed as a supplementary test in cases of doubtful or atypical reactions to the subcutaneous or other allergic tests. It is not practical for general diagnostic purposes.

(5) The subcutaneous tuberculinization of healthy animals affects the results of the complement-fixation test. Such interference may be noted as early as on the fourth day following the injection and may persist for at least six weeks, and possibly for a much longer time.

(6) There is no material difference in the reliability of the test in cattle as compared with human beings.

(7) Comparative tests with various antigens proved that one prepared with bacillary emulsion and tuberculin precipitate was the most effective.

(8) There appears to be no constancy in the presence of antibodies in tuberculous animals.

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AVAILABILITY OF POTASH IN CERTAIN ORTHOCLASE-BEARING SOILS AS AFFECTED BY LIME OR GYPSUM

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INTRODUCTION

Most soils appear to contain sufficient calcium to supply an abundance of that element so far as the actual food requirements of the crop are concerned. In the majority of cases in agricultural practice the application of lime, either as quicklime (CaO) or as the carbonate ($CaCO_3$), is made either on account of its effect upon the physical condition of the soil or to neutralize soil acids. There are cases, however, where a relatively small application of lime will accomplish beneficial effects that are not readily explainable on the basis of either of these postulates. The same is true with calcium sulphate, or gypsum ($CaSO_4.2H_2O$).

Such instances have perhaps helped to formulate the idea, apparently quite widely held, that calcium replaces potassium in the potash-bearing minerals of the soil, liberating this plant-food element. Thus, Hilgard,[1] in summarizing the important chemical effects of calcium carbonate, includes among others "the rendering available, directly or indirectly, of relatively small percentages of plant food, notably phosphoric acid and potash"; and again, in speaking of calcium sulphate, Hilgard makes the following statement:

> Being soluble in 400 parts of water, it easily penetrates downward in most soils, and in doing so effects changes in the zeolitic portions, setting free potash from silicates and thus indirectly supplying plants with this essential ingredient in a soluble form. About 200 pounds per acre is an ordinary dose.[2]

Lyon, Fippin, and Buckman speak of the action of gypsum in liberating potassium, as "a property with which it has generally been credited" . . . "the actual extent of which has never been very clearly demonstrated."[3]

André[4] reports a greatly increased solubility of the potash in microcline in the presence of calcium carbonate or calcium sulphate, the solubility being 2 and 2.5 times, respectively, that in water alone.

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[2] Hilgard, E. W. Op. cit., p. 43.

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Such reactions as may exist can not be considered to be independent of the character of the potash-bearing minerals in the soil. Prianischnikow [1] has shown with a number of different crops that marked differences exist in the availability of the potash in the various potash minerals, and that the availability corresponds approximately with the solubility of the potash of the different minerals in solutions of neutral salts. Of all the potash-bearing minerals tested, those of the feldspar group (orthoclase, microcline) showed the lowest available potash.

Blanck [2] has found that the potash in biotite and muscovite, minerals of the mica group, is more available to plants than that in orthoclase. The addition of finely ground microcline and orthoclase (equivalent to 1.6 gm. of potassium oxid) to sand (18 kgm.) having a very low potash content, resulted in little or no increase in the dry weight of oat plants, compared with plants grown in sand alone, and did not appreciably increase the potash content. An equal amount of potassium oxid applied as potassium sulphate increased the yield 70 per cent. Blanck concludes that the availability of the potash in potash-bearing minerals to plants increases proportionally to their sodium and calcium content.[3]

Many of the soils devoted to the culture of Citrus fruits in southern California have been derived from granite, pegmatite, or other rocks of this general type. Under the prevailing arid conditions the decomposition of the bedrock has been slow, and small lumps of granite, feldspar, and silica can still be found scattered plentifully through the soil.

Pegmatite and orthoclase were selected as types of the potash-bearing rocks and minerals that constitute the chief source of potassium in many of these Citrus soils. Samples collected near Riverside, Cal., were ground and passed through a 60-mesh sieve. Upon analysis by fusion, the pegmatite gave 1.50 per cent of potassium oxid and the orthoclase 12.56 per cent. The following experiments indicate that the addition of lime or gypsum to soils derived from orthoclase-bearing rocks has little or no effect, so far as the liberation of potash is concerned.

SOLUBILITY OF THE POTASSIUM IN PEGMATITE AS AFFECTED BY CALCIUM HYDRATE AND CALCIUM SULPHATE

Ten-gm. portions of pegmatite were weighed out and placed in shaker bottles with 1 liter of water, together with varying amounts of calcium hydrate (expressed as calcium oxid), as given in Table I. These mixtures

[1] Prianischnikow, D. Vegetationsversuche mit verschiedenen kalihaltigen Mineralien. *In* Landw. Vers. Stat., Bd. 77, Heft 5/6, p. 399–411, pl. 2–6. 1912.

[2] Blanck, E. Die Bedeutung des Kalis in den Feldspaten für die Pflanzen. *In* Jour. Landw., Bd. 61, Heft 1, p. 1–10. 1913.

[3] The solubility of the feldspar at temperatures above that of the soil is manifestly beyond the scope of this paper. Mention may appropriately be made, however, of a recent investigation by Stephenson, who has compared the action of various alkaline solutions on feldspars and hornblende at 100° C. and above. (Stephenson, E. A. Studies in hydrothermal alteration. Pt. I. The action of certain alkaline solutions on feldspars and hornblende. *In* Jour. Geol., v. 24, no. 12, p. 180–199, 8 fig. 1916.)

were shaken for 13 days and the clear solutions then analyzed for potash by the colorimetric method described by Cameron and Failyer.[1] The amounts of potash in these solutions is shown in Table I.

TABLE I.—*Effect of solutions of calcium hydroxid on the solubility of the potassium in pegmatite*

Solution No.	Calcium oxid in 100 c. c. of solution.	Potassium oxid in solution.
	Gm.	*P. p. m.*
1	0.00	3.1
2	.0123	3.1
3	.0246	2.5
4	.0369	3.1
5	.0492	3.0
6	.0738	3.0
7	.0984	3.1
8	[a] .1230	2.8

[a] Solid phase present.

The amount of potash recovered is so nearly uniform that these results could very well be considered to be multiplicate determinations of the potash content of the same solution. There is no evidence that the lime liberated any potash from pegmatite.

Other 10-gm. portions of pegmatite were weighed out and placed in shaker bottles. To each portion was added 1 liter of distilled water, with varying amounts of calcium sulphate, as shown in Table II. These mixtures were shaken for six days and the solutions then analyzed for potash. The amounts of potash found in solution are given in Table II.

TABLE II.—*Effect of solutions of calcium sulphate on the solubility of potash in pegmatite*

Solution No.	Calcium sulphate in 100 c. c. of solution.	Potassium oxid in solution.
	Gm.	*P. p. m.*
1	0.00	2.7
2	.0221	2.1
3	.0446	2.1
4	.0668	2.0
5	.0864	3.2
6	.1330	2.2
7	.1660	2.1
8	[a] .2100	2.8

[a] Solid phase present.

As in the case of the calcium-hydrate solutions, there is no indication that the presence of calcium sulphate led to any replacement of the potash in pegmatite by lime.

[1] Cameron, F. K., and Failyer, G. H. The determination of small amounts of potassium in aqueous solutions. *In* Jour. Amer. Chem. Soc., v. 25, no. 10, p. 1063–1073. 1903.

SOLUBILITY OF THE POTASSIUM IN ORTHOCLASE AS AFFECTED BY CALCIUM HYDRATE AND CALCIUM SULPHATE

Measurements relating to the effect of calcium hydrate on the solubility of potash in orthoclase, carried out in a similar manner to those with pegmatite, are given in Table III. Here, again, there is no indication that the presence of calcium affects the solubility of the potash.

TABLE III.—*Effect of solutions of calcium hydrate on the solubility of the potash in orthoclase*

Solution No.	Calcium oxid in 100 c. c. of solution.	Potassium oxid in solution.
	Gm.	*P. p. m.*
1	0.00	10.8
2	.0103	8.6
3	.0207	12.0
4	.0414	8.2
5	.0621	9.1
6	.0828	12.6
7	.1035	12.1
8	[a].1242	9.6

[a] Solid phase present.

The results of similar determinations of the solubility of the potassium in orthoclase in the presence of solutions of calcium sulphate of varying concentration are presented in Table IV. A different sample of orthoclase was used in this case from that employed in the calcium hydroxid series, which accounts for the difference in solubility of the feldspar in distilled water in the two series. As before, there is no indication that potash is being replaced by lime. On the other hand, the results indicate that increasing the concentration of the calcium sulphate depresses the solubility of the potash.

TABLE IV.—*Effect of solutions of calcium sulphate on the solubility of the potash in orthoclase*

Solution No.	Calcium sulphate in 100 c. c. of solution.	Potassium oxid in solution.
1	0.00	6.0
2	.018	4.5
3	.035	3.6
4	.070	3.6
5	.105	1.2
6	.140	.8
7	.175	1.3
8	[a].210	.5

[a] Solid phase present.

SOLUBILITY OF THE POTASH IN SOILS OF THE RIVERSIDE AREA AS INFLUENCED BY CALCIUM HYDRATE AND CALCIUM SULPHATE

A large sample of virgin soil from the site of the new University of California Citrus Experiment Station at Riverside was collected, dried in the sun, and passed through a 1-mm. sieve. Portions of this soil of 100 gm. each were weighed out and 500 c. c. of distilled water added, together with varying amounts of calcium hydroxid, as shown in Table V. The mixtures were shaken until the solutions had reached equilibrium. The amounts of potash found in the solutions are given in Table V. As in the case of the pegmatite and orthoclase experiments, the addition of calcium hydroxid does not modify the solubility of the potash in this soil sufficiently to be detected in the analyses.

TABLE V.—*Effect of solutions of calcium hydrate on the solubility of the potash in soil from the Citrus Experiment Station site*

Solution No.	Calcium oxid in 100 c. c. of solution.	Potassium oxid in solution.
	Gm.	*P. p. m.*
1	0.00	26.4
2	.0103	26.4
3	.0207	28.8
4	.0414	28.8
5	.0621	24.0
6	.0828	27.6
7	.1035	25.2
8	[a].1242	27.6

[a] Solid phase present.

The solubility of the potash in the soil from the Citrus Experiment Station site in the presence of varying amounts of calcium sulphate was also determined. The concentration of the calcium-sulphate solutions and the amount of potash found in solution are given in Table VI.

TABLE VI.—*Effects of solutions of calcium sulphate on the solubility of the potash in soil from the Citrus Experiment Station site*

Solution No.	Calcium sulphate in 100 c. c. of solution.	Potassium oxid in solution.
	Gm.	*P. p. m.*
1	0.00	24
2	.017	24
3	.034	26
4	.068	29
5	.102	26
6	.136	26
7	.170	29
8	[a].210	26

[a] Solid phase present.

The average variation (1 part in 25) in the potash content is well within the experimental error, when the lack of uniformity in the soil and the analytical difficulties are considered. There is no evidence that the addition of calcium sulphate modified to a measurable degree the solubility of the potash in this soil.

Another soil from the Oatman tract, about 7 miles from Riverside, was treated with calcium-sulphate solutions in the same way. This soil, which had been under cultivation for a number of years, was more granitic in character and less weathered than the virgin soil. The potash solubility in varying concentrations of calcium sulphate is shown in Table VII. Here, again, there is no indication of any replacement of potash by the lime; but, on the contrary, the addition of calcium sulphate depresses the solubility of the potash, as was found in the case of orthoclase (Table IV).

TABLE VII.—*Effect of solutions of calcium sulphate on the solubility of potash in Oatman soil*

Solution No.	Calcium sulphate in 100 c. c. of solution.	Potassium oxid in solution.
	Gm.	*P. p. m.*
1	0.00	8.6
2	.017	8.6
3	.034	8.0
4	.068	4.2
5	.102	2.2
6	.136	2.2
7	.170	2.4
8	[a].210	4.2

[a] Solid phase present.

LeClerc and Breazeale[1] have shown that the sprouting seedling of wheat manifests a strong demand for potash, entirely out of proportion to its demand for nitrogen and phosphoric acid. When potash is present in a readily available form, the young wheat seedlings, during the first three weeks of growth, will often take up an amount of potash (K_2O) equivalent to 8 per cent or more of their dry weight. This avidity of the plant for potash was made use of in the following manner: Wheat seeds were sprouted on perforated aluminum disks floating in large pans. Each pan contained about 1,000 seeds. When the shoots had reached a length of about 2 cm., the plants were taken up, a few at a time, and transplanted to other disks, floating in the various solutions listed in Table VIII. In this way all the unsprouted seeds were eliminated, which would otherwise have become a source of potash. The solutions in the pans were stirred frequently and the plants were

[1] LeClerc, J. A., and Breazeale, J. F. Translocation of plant food and elaboration of organic plant material in wheat seedlings. U. S. Dept. Agr. Bur. Chem., Bul. 138, 32 p., 2 fig. 1911.

allowed to grow for varying periods, ranging from 5 to 12 days. Plant samples were then withdrawn and analyzed for potash by the official method. Table VIII shows the treatment and the percentage of potassium oxid in each lot of plants, expressed in terms of the dry weight.

TABLE VIII.—*Potash absorbed by wheat seedlings from orthoclase and soil solutions as affected by calcium sulphate.*

Series No.	Treatment.	Percentage of potassium oxid in dry plants.
1	10 gm. of orthoclase, 2,500 c. c. of carbon-treated water	1.10
1	Same, saturated with calcium sulphate	.95
2	10 gm. of orthoclase, 2,500 c. c. of carbon-treated water	1.84
2	Same, saturated with calcium sulphate	1.72
3	40 gm. of orthoclase, 2,400 c. c. of carbon-treated water plus 200 p. p. m. NO_3 and 200 p. p. m. P_2O_5	2.56
3	Same, saturated with calcium sulphate	2.57
4	50 gm. of Oatman soil, 2,500 c. c. of carbon-treated water	1.75
4	Same, saturated with calcium sulphate	1.35

The period of growth was varied so that the dry weight of the plants in the different series was not the same, which accounts for the differences in the potash content of the various cultures when expressed in percentage of the dry weight. In the third series 200 p. p. m. of NO_3 as $NaNO_3$ and 200 p. p. m. of P_2O_5 as Na_2HPO_4 were added to balance the nutrient solution.

To insure the solution of the potash, the solutions containing finely ground orthoclase in series 3 were shaken up for four days before they were used for culture purposes. Analyses of the resulting solutions at the time the cultures were started showed 9.6 p. p. m. of potassium oxid where gypsum was absent and 6 p. p. m. where gypsum was present. Such potash concentrations are sufficient for the nutrition of plants, as the potash content of the soil solution in humid soils seldom exceeds 10 p. p. m. It will be shown in another paper that wheat plants can readily take up potash and other salts in concentrations of 1 p. p. m. or less.

If the calcium sulphate had liberated any additional potash from the feldspar, the seedling plants would have taken it up, and this would have been shown by an increase of the potash content of the plant ash. In none of the three series of cultures, however, was the potash content of the plants increased by the addition of gypsum to the solution.

In the Oatman soil (series 4) the addition of gypsum decreased the absorption of potash by plants. It will be recalled that the analyses of the solutions also showed that the use of gypsum depressed the solubility of the potash in this soil (Table VII).

SUMMARY

It is stated in agricultural treatises that the application of lime to a soil liberates potash from the soil minerals. This subject is of special import to the Citrus industry of southern California in which commercial fertilizers are extensively used and heavy applications of lime and gypsum are sometimes made.

Samples of pegmatite and orthoclase were collected near Riverside, Cal., representing, respectively, types of the potash-bearing rock and mineral from which many of the Citrus soils appear to be derived. These samples were finely ground and shaken for a number of days with aqueous solutions of calcium hydroxid and of calcium sulphate in graduated concentrations. The calcium-hydrate solutions did not modify the solubility of the potassium in either pegmatite or orthoclase. Gypsum solutions depressed the solubility of the potassium in orthoclase, the quantity of potash in solution decreasing progressively as the concentration of the calcium sulphate increased.

Similar tests were made upon a virgin soil of a granitic type from the experiment station near Riverside, Cal. The solubility of the potash was not measurably different in distilled water and in solutions of calcium hydrate or calcium sulphate.

The addition of calcium sulphate to a Citrus soil which had been under cultivation for some time and which was more granular and less weathered than the virgin soil, decreased the solubility of the potash.

The potassium content of wheat seedlings was practically the same when grown (1) in water containing finely ground orthoclase and (2) in a saturated calcium-sulphate solution containing the same quantity of orthoclase.

Similar experiments in which a Citrus soil was used instead of orthoclase showed a decreased absorption of potassium by wheat seedlings in the presence of calcium sulphate.

In brief, the experiments indicate that the availability to plants of the potash in soils derived from orthoclase-bearing rocks is not increased by the addition of lime or gypsum. In some instances a marked depression of the solubility of the potash in the presence of gypsum was observed. These conclusions are based both on the results of the analyses of the solutions and on the measurement of the potash content of wheat seedlings grown in the solutions.